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(54) Title: ULTRASMALL NON-AGGREGATED POROUS PARTICLES ENTRAPPING GAS-BUBBLES

(57) Abstract

Ultra-small, substantially non-aggregated, non-crystalline particles of predetermined uniform size wich, when suspended in a liquid, contain entrapped gaseous bubbles are disclosed. These gaseous bubble particles are prepared by simultaneous co-precipitation of two compounds wherein one compound is substantially more soluble than the other in a given vehicle. When this vehicle is used for washing the co-precipitated particles, part of the soluble material is dissolved leaving a porous matrix. The porous particles then are dried and stored. The porous particles, which can be resuspended immediately prior to use, contain entrapped gas in the evacuated crevices or pores which is not displaced for a period of time because of surface tension of the suspending vehicle. The ultrasmall porous particles can be used as ultrasound contrast agents, such as ultrasound contrast agents in the blood vessels and soft tissue, including the liver, spleen, heart myocardium, kidney and brain of an animal, such as a human. The ultrasmall porous particles also can be used dry for applications where reduced weight is desirable.

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ULTRASMALL NON-AGGREGATED POROUS PARTICLES ENTRAPPING GAS-BUBBLES

Field of the Invention

The present invention relates to ultra-small, substantially non-aggregated porous particles of predetermined uniform size which, when reconstituted contain entrapped gas bubbles. The ultrasmall porous particles are suitable for use as fillers and the like and as ultrasound contrast agents when suspended in a suitable ultrasound liquid. The present invention further relates to methods of making and using such ultrasmall, uniform porous particles.

Background of the Invention

Particles of compounds having low solubility in a dispersing medium are commonly used in a wide variety of applications, including pharmaceuticals, ceramics, paints, inks, dyes, lubricants, pesticides, insecticides, fungicides, fertilizers, chrom-

atography columns, cosmetics, lotions, ointments, and detergents. Aqueous dispersions of particles are used in many cases to avoid hazards such as flammability and toxicity associated with organic solvents. Such dispersions typically have a broad range of particle size.

In many cases, product performance is improved by controlling the particle size distribution. In general, smaller particles of a compound provide a more uniform dispersion and will dissolve faster than larger particles of the same compounds. Control of particle size is, therefore, important in controlling the rate of solubilization.

Many drugs have been formulated as particles for sustained-release following oral, aerosol, subcutaneous, intramuscular, or other routes of administration. Particle size is one important factor affecting the release rate of these drugs. Those skilled in the art can discern other examples for using particle size to control product performance for the substances listed above.

Drugs that are insoluble in water can have significant benefits when formulated as a stable suspension of particles of less than three microns diameter. In this particulate form, the drug can be injected intravenously, circulate in blood, and be preferentially accumulated in, for example, the

reticuloendothelial system, where it can facilitate normal reticuloendothelial functions such as detoxification. Alternatively, the drug can reside in the reticuloendothelial cells where it is stored until solubilized or metabolized into an active form which circulates in blood to other tissues for efficacy. This "slow" release of active drug can provide more constant drug concentrations in plasma over a period of hours, days, weeks, or months, resulting in improved therapeutic efficacy. Biodegradable particles which are radiopaque or labelled with a radioisotope are useful for diagnostic imaging of organs, such as liver and spleen, with high concentrations of fixed reticuloendothelial cells.

Solid biodegradable particles also can be useful for diagnostic ultrasound imaging of the liver and spleen with ultrasound. These particles can be effective if they have a density or compressibility significantly different from surrounding tissue, thereby producing an impedance mismatch responsible for backscatter enhancement. Since tumors and other lesions generally do not contain these fixed reticuloendothelial cells, particles are not accumulated in these lesions so only tissue parenchyma backscatter is enhanced creating a larger difference in echogenicity between parenchyma and lesion thereby facilitating lesion

detection and diagnosis by ultrasound. See, for example, Parker, K.J. et al.: Ultrasound in Med. & Biol., 13(9): 555-566 (1987).

By far the simplest form of ultrasound contrast agents is free gas bubbles. Such bubbles may preexist in the liquid vehicle, or may be introduced via cavitation during the injection phase. Whatever the mechanism may be, it appears that many liquids, when rapidly injected into ducts or vessels, are capable of generating a quantity of air bubbles which may produce sufficient echoes to cause partial or complete intraluminal sonographic opacification.

The first report on the use of free gas bubbles appears to be that of Gramiak, R. and Shah, P.J.: Investigative Radiology, 3:356-366 (1968). They reported that they obtained anatomic validation of the aortic origin of cardiac echoes by means of direct physiological saline injection during continuous echocardiographic recording. The injection produced a cloud of echoes which was delineated by the parallel signal of the aortic root. See also, for example, U.S. Patent No. 4,276,885. Kremkau, F.W. et al.: Am. J. Roentgend., 3:159 (1968) also reported that they obtained intracardiac echoes from saline injection and from injection of autologous blood. They demonstrated that air bubbles may be

generated during the injection process itself. Ziskin, M.C. et al.: Investigative Radiology, 7:500-505 (1972) reported using a variety of liquids, such as renografin, carbonated water, and ether (which boils at body temperature) to demonstrate the presence of echoes in all cases, detected by enhanced Doppler signals from arteries. In recent years, numerous investigators such as Chiang, C.W. et al.: Chest, 89(5):723-726 (1986), Rizayev, M.N. and Azatyan, T.S.: Heart J., 10(6):1308-1310 (1985), Feinstein, S.B. et al.: J. Am. Coll. Cardiol., 3(1):14-20 (1984), Konodo, S. et al.: J. Am. Coll. Cardiol., 4:149-156 (1984), Ten-Cate, F.J. et al.: J. Am. Coll. Cardiol., 3(1):21-27 (1984), Maurer, B. et al.: Circulation, 69(2):418-429 (1984), Armstrong, W.F. et al.: J. Am. Coll. Cardiol., 2(1):63-69 (1983), Tei, C. et al.: J. Am. Coll. Cardiol., 3(1):39-46 (1984), Armstrong, W.F. et al.: Circulation, 66(1):166-173 (1982), Meltzer, R.S. et al.: Br. Heat J., 44(4):390-394 (1980a), Meltzer, R.S. et al.: J. Clin. Ultras., 9(3):127-131 (1981), Wise, N.K. et al.: Circulation, 63(5):1100-1103 (1983), Meltzer, R.S. et al.: Ultrasound Med. Biol., 6(3):263-269 (1980b), have reported the use of indocyanine green for opacification of the common bile duct in cholangiography. Presumably, microscopic air bubbles

contained in the liquid or generated during the injection phase is responsible for the observed effects. Meyer-Schwickerath, M. and Fritzsch, T.: Ultraschall Med., 7:34-36 (1986) have reported urologic applications of a new commercial agent which incorporates solid particles as microbubble carriers.

While free gas bubbles are extremely efficient scatterers of sound energy, their utility is limited by the fact that they are effectively removed by the lungs or by pressure changes in the heart. Thus, it is impractical to use microbubbles to elicit contrast in the soft tissue via venous injection.

In an effort to overcome some of the limitations of free gas bubbles, encapsulated gas bubbles were manufactured and injected directly into the carotid artery in tumor bearing rabbits. See Carroll, B.A. et al.: Investigative Radiology, 15(3):260-266 (1980). These consisted of nitrogen gas trapped in 80 micron gelatin capsules. Carroll et al. report ultrasonic enhancement of tumor rims in rabbits with VX2 carcinoma. The large size of these particles did not allow their administration in the peripheral circulation. Unfortunately, the manufacturer of small (2-3 microns), gas filled capsules which could clear the lungs is difficult due to the extreme

thinness of the capsule wall through which gas diffuses.

As another alternative, U.S. Patents, No. 4,442,843, No. 4,657,756 and No. 4,681,119, disclose the use of aggregates as carriers of gas to produce microbubbles in blood to alter the transmission characteristics thereof to electromagnetic and sonic waves transmitted therethrough. Unfortunately, because the aggregates are of such a large size, i.e., on the order of between about 20-250 microns, it is difficult if not impossible for such aggregates to travel past the lungs and heart, thereby limiting their usefulness as ultrasound contrast agents. In addition, because the solid materials from which the aggregates are formed will generally solubilize in body fluids over a short period of time, their ability to enhance ultrasound images in the lungs and heart is short-lived. Moreover, the individual particles from which the aggregates are formed have little bubble generating capacity in their unaggregated form.

Consequently, there is a demand in the ultrasound industry for a contrast agent which offers enhanced gas bubble echogenicity with good long-term stability for arterial and organ ultrasound image enhancement following intravenous injection.

Summary of the Invention

In brief, the present invention alleviates and overcomes certain of the above-mentioned problems and shortcomings of the present state of the art through the discovery of novel ultrasmall, substantially non-aggregated, non-crystalline porous particles of substantially uniform size which, when reconstituted, contain entrapped gas bubbles, and methods of making and using same.

The novel ultrasmall, non-aggregated porous particles of the instant invention are uniquely suited for use as ultrasound image enhancers and for ultrasound measurements, such as Doppler techniques, when reconstituted with suitable physiologically acceptable liquids. Quite amazingly, the novel ultrasmall non-aggregated porous particles provide unique gas bubble echogenicity with good long-term stability for arterial and organ ultrasound image enhancement following intravenous injection. Even more amazingly, the ultrasmall porous particles of the instant invention are able to accomplish this without having to form aggregates in order to develop microbubbles to enhance ultrasound imaging.

It has also been discovered, and quite surprisingly, that the novel ultrasmall, non-aggregated porous particles of the instant invention produce enhanced and long-term ultrasound back

scatter as a result of gas bubbles, such as air or helium bubbles, being trapped within their solid matrices or pores when they are suspended in a liquid. It is believed that the solid matrices or pores provide the stability for the unique sustained echogenicity which heretofore has not been achieved by the ultrasound contrast agents presently available. And, because the novel ultrasmall porous particles of the instant invention have virtually no tendency to aggregate, they are uniquely suited for use as contrast agents to enhance ultrasound images in the blood vessels and soft tissue or organs throughout an animal. In other words, because the novel ultrasmall porous particles have the capacity to circulate throughout the body of an animal, their suitability as ultrasound contrast agents greatly extends beyond the lungs and heart into, for example, the liver, spleen, heart myocardium, kidney, brain and the like.

In accordance with the present invention, the ultrasmall, porous particles of the instant invention are believed to be stable at ambient temperatures, have little to no tendency to aggregate, and are non-toxic and physiologically acceptable when introduced into the bloodstream of living beings, such as humans. The size of the ultrasmall, porous particles of the present invention typically

range up to about 10 microns, and preferably from about 0.01 microns to about 5.0 microns, and more preferably from about 0.1 microns to about 2.0 microns.

Preferred ultrasmall, substantially non-aggregated, non-crystalline porous particles of the instant invention are iodipamide ethyl ester particles having a substantially uniform mean diameter on the order of, for example, about 0.5 up to about 2 microns and the ability to entrap gas bubbles within their pores after resuspension in a liquid vehicle.

In accordance with a further feature of the instant invention, the ultrasmall porous particles of substantially uniform size are made by, first, preparing a solution of two separate solid compounds in a suitable solvent for the two compounds, second, infusing a precipitating liquid into the solution at a temperature between about -50°C and about 100°C and at an infusion rate of from about 0.01 ml/min. to about 3000 ml/min. per unit volume of 50 ml, the two solid compounds having essentially little solubility in the precipitating liquid and the solvent being miscible in the precipitating liquid, so as to produce a suspension of precipitated solid compounds in the form of substantially non-aggregated particles with a substantially uniform mean particle diameter selected from the range of up to about 10

microns, such that the particle size is directly related to the solution temperature and inversely related to infusion rate, third, separating the co-precipitated particles from the solvent, and, fourth, washing the co-precipitated particles with a washing liquid which serves to selectively solubilize and remove the second compound as well as any remaining residual solvent thereby producing particles consisting of a porous matrix consisting of only, or mostly, the first compound. For example, when producing ultrasmall porous particles, such as ultrasmall porous iodipamide ethyl ester (IDE) particles, in accordance with the instant invention, the IDE porous particles formed after the washing step are believed to be mostly IDE, but the matrix of the IDE particles may include, for instance, some iodipamic acid (IDA). The ultrasmall "porous" particle suspension is then exposed to gas, for example, at increased pressure, or dried to remove as much moisture as possible and to permit the porous particles to entrap gas within their porous matrices upon suspending the particles in liquid vehicles. The ultrasmall porous particles of the instant invention are much less dense than pure solid particles formed alone following the same procedure.

It should be appreciated by those versed in this art that when the dried ultrasmall, porous particles are reconstituted or suspended in a suitable liquid, they are completely redispersed, but now a small amount of air or other gas such as helium has been entrapped in the particle crevices or pores where the second compound initially was present. The entrapped air, crevices or pores is believed to remain for several hours, even after resuspension in a liquid vehicle. Moreover, it is believed that because the crevices or pores are so small, the surface tension of the suspending liquid does not permit rapid filling. Consequently, such reconstituted suspensions are uniquely suited for use as ultrasound contrast materials because the echogenic gas bubbles trapped within the solid porous particles are stabilized, even against pressure changes in the heart which typically destroys other competitive ultrasound contrasting agents currently available.

In practicing the methods to produce the ultrasmall porous particles of the instant invention, a preferred weight ratio of the more soluble to the less soluble compound in the washing solution is from about 2:1 up to about 10:1. In addition, the amount of the more soluble compound dissolved and removed during the washing of the particles can

be from about 10% to about 100% of the amount of the more soluble compound present in the particles after precipitation, but before washing.

In one preferred embodiment, the less soluble compound in the washing solution is iodipamide ethyl ester (IDE) and the more soluble compound in the washing solution is iodipamic acid (IDA). The precipitating liquid is water at about pH 5 and the washing liquid is aqueous 0.1% PVP at about pH 11. When washing the co-precipitated particles in accordance with the methods of the instant invention, washing can be continued until most or all IDA has been removed but most or all of the IDE remains which, for example, is preferably at a pH of about 3.3 to about 3.4 when producing ultrasmall porous IDE particles. When washing is stopped at a pH of about 3.3 to 3.4, it is believed that the yields and echogenicity of the ultrasmall porous IDE particles are enhanced.

In accordance with a further feature of the present invention, the ultrasmall, porous particles may be coated with various substances, such as human serum albumin or selected antibodies, to alter the surface properties of the particles to improve, for example, their biocompatibility or their ability to target a desired site.

Accordingly, it can now be appreciated by those versed in this art that the present invention provides a solution to the ultrasound art that has long sought to overcome the shortcomings associated with the ultrasound contrast agents and methods available heretofore.

The above features and advantages of the present invention will be better understood with reference to the FIGS., Detailed Description and Examples set out hereinbelow. It will also be understood that the ultrasmall, porous particles and methods of this invention are exemplary only and are not to be regarded as limitations of this invention.

Brief Description of the FIGS.

Examples of the present invention will now be more fully described, with reference to the accompanying FIGS., wherein:

FIG. 1 depicts raw backscatter values (RMS of *in vitro* bubble/IDE particle suspensions at 5.2 mg/ml plotted versus the weight ratio of iodipamic acid (IDA) to iodipamide ethyl ester (IDE) in a formulation mixture before dissolving away the IDA.

These data demonstrate increased echogenicity (porosity) with higher ratios of IDA/IDE;

FIG. 2 depicts raw backscatter values (RMS) of *in vitro* bubble/IDE particle suspensions at 5.2,

9.1 and 13.1 mg/ml plotted versus time after mixing with bovine plasma. These data demonstrate increased backscatter with increased particle concentration. These data also demonstrate the extremely long persistence of this backscatter after mixing with bovine plasma compared with other bubble agents which survive only seconds to minutes;

FIG. 3 depicts raw backscatter values (RMS) of in vitro bubble/IDE particles at 5.2 mg/ml and standard solid IDE particles at 12.2 mg/ml plotted versus time. These data demonstrate the increased echogenicity obtained with bubble/IDE particles compared with that for solid IDE particles. This enhanced echogenicity is observed even though the bubble/IDE particles are at less than half the concentration of the solid IDE particles;

FIG. 4 depicts B-scan image at 5 MHz of normal rabbit liver with gall bladder before infusion of the bubble/IDE contrast agent;

FIG. 5 depicts B-scan image at 5 MHZ of normal rabbit liver with gall bladder, but 60 minutes following intravenous infusion of the bubble/IDE contrast agent. The enhancement of the parenchymal echogenicity was observed for at least 120 minutes after contrast administration;

FIG. 6 is a graph of infusion rate (ml/min.) (of aqueous precipitating liquid) as a function of the product of stir rate (rpm) and total volume (liters) of the organic solution at a constant temperature; the relationship: aqueous infusion rate (ml/min.) = 23 + 0.14 [stir rate (rpm) X volume organic solution(l)] defines the parameters for production of iodipamide ethyl ester particles of one micron diameter at a constant temperature (4°C) and in dimethylsulfoxide/ethanol;

FIG. 7 is a graph showing iodipamide ethyl ester particle size as a function of temperature at a constant ratio of infusion rate of aqueous precipitating liquid to [stir rate (rpm) X volume of organic solution];

FIG. 8 is a graph demonstrating the effect on particle size of varying the infusion rate of aqueous precipitating liquid at constant temperature and stirring rate of an iodipamide ethyl ester solution; and

FIG. 9 depicts raw backscatter values (RMS) of <u>in vitro</u> bubble-IDE particle suspensions at 5.2 mg/ml. These data demonstrate that backscatter increases linearly with concentration.

Detailed Description of the Invention

By way of illustrating and providing a more complete appreciation of the present invention and many of the attendant advantages thereof, the following detailed description is provided concerning the novel ultrasmall, substantially non-aggregated, non-crystalline porous particles, and methods of making and using such particles.

This invention concerns the preparation of non-aggregated porous particles of a predetermined uniform size. One aspect of the invention concerns the preparation of uniform particles of a predetermined size in a vehicle in which the concentration of the compound in the vehicle is greater than the solubility of the compound in that vehicle. The particles are formed by a carefully controlled precipitation of the compound into a suitable precipitating liquid from a solvent in which the compound is soluble.

A second aspect of this invention is the simultaneous co-precipitation of two compounds having significantly different solubilities in designated washing solutions such that the more soluble compound can be dissolved and removed from the less soluble compound during the washing of the co-precipitated particles. The porous particles then can be coated to change the surface properties

if so desired. The porous particles can then be dried to remove almost all moisture from the particles leaving porous particles with lower density. Such particles in dry form can be useful for high strength low weight materials.

Particles which are about one micron diameter or smaller have pores so small that they do not fill rapidly with liquid when the particles are rewetted. In this way it is possible to entrap air (or other gas) in the particles. Such particles can be useful as echogenic ultrasound contrast materials.

An important principle underlying this invention is the differential solubilities of the two compounds chosen for co-precipitation. For example, an ester and the acid from which it is synthesized may both be insoluble in aqueous solutions at, for example, pH 5-7. However, the acid may have significantly higher solubility at pH 10-11 such that washing at basic pH dissolves the acid leaving porous particles consisting solely, or mostly, of the ester compound. Other acid derivatives, such as amides can be substituted for the ester compound recognizing that the wash solution may have to be at a lower pH to be effective.

Similarly, two compounds could be chosen for washing with an organic solvent as long as the

two compounds have significantly different solubilities in that solvent.

This invention can be practiced utilizing compounds which differ in solubility in a given wash solution by orders of magnitude. The difference in solubility, however, can be small - a factor of two or less - but washing conditions must be adjusted to create the porous particles.

Varying the ratio of more-soluble to less soluble components can alter the properties of the resultant particles. For example, varying the ratio of iodipamic acid (IDA) to iodipamide ethyl ester (IDE) from 2:1 to 8:1 significantly increases the echogenicity of the resultant particles, as shown in FIG. 1. These data illustrated in FIG. 1 were acquired at 4.7 mg/ml.

Varying the concentration of particles also affects the echogenic properties of a suspension. As shown in FIG. 2, the RMS backscatter of particles in plasma increased from 0.4 to 1.2 when the concentration of particles increased from 5.2 to 13.1 mg/ml.

The ultrasmall porous particles of the instant invention can be coated with substances to alter their surface properties. For example, coating the particles with serum albumin can improve the biocompatibility of such particles. Coating with

antibodies may improve the targeting of particles to a desired site. Coatings altering the wetability, charge, or permeability of the particle surface of such particles may be applied prior to drying to achieve the desired surface characteristics.

Drying can be accomplished by any of a number of techniques known to one skilled in the art. Vacuum, spray, or lyophilization may be utilized depending on the compound(s) and application for these particles.

After drying, the particles can be reconstituted with any liquid in which the particles are not soluble. For ultrasonic contrast agents, the liquid generally will be water or some other suitable aqueous solution. Other liquids may be appropriate for other applications.

Methods other than drying can be utilized to introduce gas into the porous particles. For example, supersaturation and rectified diffusion under acoustic irradiation can utilize the porous particles as nuclei for gas cavity formation in situ.

The preferred IDE/IDA particles are believed to be useful for diagnostic imaging with both computer tomography (CT) and ultrasound. The iodine in the IDE solid matrix is the effective attenuator for CT while the entrapped air is the effective scatterer for ultrasound. Similar particles could

be useful for magnetic resonance imaging as well. This would be possible by reconstituting the dried particles with a liquid containing a paramagnetic material, such as gadolinium chelates. After allowing sufficient time for equilibration of the paramagnetic material into the pores of the particles, the particles could be useful for enhanced magnetic resonance imaging of tissues, e.g. liver, in which the biodistribution of the entrapped paramagnetic material is controlled by the particles rather than the bulk liquid biodistribution. It is believed that this can be accomplished by suspending the dried porous particles in a paramagnetic material, such as gadolinium EDTA (Gd EDTA). For example, once the porous partices have been suspended in gadolinium EDTA, they can be introduced into an animal in an effective amount such that the porous particles and Gd EDTA will accumulate in the phagocytic cells in the liver, while the remaining Gd EDTA will distribute normally and clear quickly from the animal. The Gd EDTA accumulated in the liver will remain in the phagocytic cells for a period of time to enhance the liver parenchyma during imaging. Tumor and metastatic cells will, however, not be enhanced upon imaging thereby improving their detection. Other combinations,

the precipitating liquid. Thus, compounds having essentially little aqueous solubility, i.e., compounds which have an aqueous solubility of less than one part in ten thousand, may be precipitated in an aqueous solution in order to obtain an excellent yield. Compounds which are more water-soluble can also use an aqueous precipitating liquid. However, the higher the solubility of the compounds, the greater the probability that some of the compounds will dissolve in the aqueous phase and transform to the more stable crystalline state. Also, redissolution in the aqueous phase can lead to a broadening of the particle size distribution. For these reasons, it is preferred that an aqueous precipitating liquid be used for compounds having a water-solubility of less than one part in ten thousand.

It has been found that it is possible to prepare suspensions of compounds which are poorly soluble in aqueous solutions, i.e., have a solubility from about one part per ten thousand to about one part per one hundred which provide excellent yields by using an acceptable precipitating liquid in which the compounds have even less solubility than water. The difference in the solubility of the compounds in water as compared to the precipitating liquid need not be large in order to be significant in terms of

yield. In order to make particles of a uniform and predetermined size, a solution of the solid compounds in a suitable solvent is prepared. The solution may be diluted with a non-solvent that does not cause the compounds to precipitate. A precipitating liquid is also prepared, preferably with a surfactant, in sufficient quantity to both coprecipitate the compounds and stabilize the resulting suspension of particles of the compounds against aggregation. The precipitating liquid may be used alone when compounds which do not aggregate are used. The precipitating liquid is infused into the solution in which the compounds are dissolved under carefully controlled conditions, including: the rate of stirring of the organic solution, the rate of infusion of the aqueous solution, the volume of the organic solution and the temperature of the solutions and the suspension. The precipitating liquid may be infused, for example, through a needle of standard gauge.

In investigations of varying parameters to adjust for particle size, three usable relationships were discovered: (1) diluting the solution with more of the non-solvent produces larger particles, and diluting with less of the non-solvent produces smaller particles; (2) higher temperatures of the

solution during precipitation produce larger particles, and lower temperatures of the solution during precipitation produce smaller particles; and (3) at a given stirring rate of the organic solution, faster infusion rates of precipitating liquid produce smaller particles while slower infusion rates produce larger particles.

When the co-precipitation is complete, the uniformly sized particles are washed to remove the solvent, i.e. by centrifugation, filtration, etc, and the soluble compound to produce the porous particles of the instant invention. In most cases, the particles should be separated from the solvent quickly to prevent transformation to a crystalline form.

Aqueous precipitating liquids are useful for many compounds, including but not limited to organic compounds such as iodipamide ethyl ester, iothalamate ethyl ester, iosefamate ethyl ester, 2,2', 4 4'-tetra-hydroxybenzophenone, RS nitrocellulose, progesterone, beta-2,4,6-triiodo-3-dimethyl formamidinophenyl propionic acid ethyl ester, N-(trifluoroacetyl) Adrimycin 14 valerate, 1,2 diaminocyclohexane malinate platinum (II), norethisterone, acetyl salicylic acid, wafarin, heparin-tridodecyl methyl ammonium chloride complex, sulfamethoxazole, cephalexin, prednisolone acetate,

diazepam, clonazepam, methidone, naloxone, disulfiram, mercaptopurine, digitoxin, primaguine, mefloquine, atropine, scopolamine, thiazide, furosemide, propanalol, methyl methacrylate, poly methyl methacrylate, 5-fluorodeoxyuridine, cytosine arabinoside, acyclovir, and levonorgestrel; and inorganic compounds such as aluminum chloride hexahydrate, the oxides of iron, copper, manganese and tin.

Compounds which are better suited for precipitation using a non-aqueous precipitating liquid include organic compounds such as mitindomide, hydrolytically unstable compounds such as isopropylpyrrolizine (IPP, or carbamic acid, (1-methylethyol)-,(5-(3, 4-dichlorophenol)-2, 3-dihydro-1,H-pyrrolizine-6-,7-diyl) bis(-methylene ester); and inorganic compounds such as iron citrate, iron iodate, calcium pyrophosphate, calcium salicylate, platinum dichloride and sodium pyrophosphate.

The first step is to prepare a solution of two compounds, one compound being of interest, in a suitable solvent for the compounds. This can occur by simply dissolving the compounds in the solvent of choice.

The solvent is chosen to suit the compounds. For example, dimethylformamide (DMF) is a

solvent for iothalamate ethyl ester (IEE) and iosefamate ethyl ester (IFE), and dimethylsulfoxide (DMSO) is a solvent for iodipamide ethyl ester (IDE) and IEE. DMSO is also a suitable solvent for compounds such as mitindomide. Another suitable solvent for many compounds, and especially IPP, is tetrahydrofuran (THF).

The solution is then optionally diluted with a non-solvent that does not cause the compounds to precipitate. The non-solvent causes greater dispersion of the dissolved molecules of the compounds in the liquid phase. Greater dilution of the solution with non-solvent produces larger particles, and less dilution of the solution with non-solvent produces smaller particles.

The non-solvent should not precipitate the compounds when it is added to the solution. Lower aliphatic alcohols, such as ethanol, are effective non-solvents for solutions of IDE and IEE in DMSO. For the ethyl esters of triiodobenzoic acid, proportions of non-solvent to solvent at a ratio of 2 or more can produce 1 to 3 micron sized particles (depending on other parameters); and ratios of less than 2 can produce submicron particles, at least as applied to DMSO solutions diluted with ethanol.

To co-precipitate the compounds from the solution in a desired particle size, preferably a

solution of a surfactant is prepared in sufficient quantity to effect complete precipitation of the compounds and to stabilize the resulting suspension of particles of the compound against aggregation. The surfactant provides the stabilization against aggregation, while a suitable precipitating agent causes the co-precipitation of the compounds. Presence of extra surfactant solution is advisable to ensure stabilization so that the co-precipitated particles suspended in liquid do no aggregate, forming agglomerates of an improperly large size. While surfactants are used in most cases, some compounds appear to form stable, substantially non-aggregated particles without the use of surfactants. Examples of such non-aggregating compounds are certain heparin complexes.

It is thought that particles with relatively high surface charge are less likely to require surfactant in the precipitating solution. The surface charge of a particle is sometimes referred to as its zeta potential, a measurement of charge which falls off with distance. There may be a threshold zeta potential above which no surfactant is needed, but below which, surfactant is needed to keep the precipitating particles from aggregating. The zeta potential is directly correlated with the polarity or net charge of a compound. Thus, the

need for surfactant in the precipitating solution may be predicted from the extent of the charge or polarity of the compound employed in the method of the invention. For example, heparin complexes are highly charged, and form stable non-aggregated particles when precipitated with water.

Generally, such a theory notwithstanding, empirical methods will suffice; that is, a co-precipitation may first be performed with water, and if aggregation occurs, then a co-precipitation in the presence of surfactant is indicated. Surfactants are chosen for their compatibility with the compounds and their ability to stabilize a suspension of compound particles. For work with IEE and IDE drugs, a solution of 5% polyvinylpyrrolidone (C-30), 0.1% poly-vinylpyrrolidone (C-15), or 0.1% human serum albumin is preferred. Also 0.1% Pluronic F-68, [Poloxamer 188, a poly(oxyethylene-co-oxypropylene) polymer], a 0.33% gelatin, 0.33% gelatin plus 0.6% Hetastarch, 0.33% gelatin plus 0.002% propylene glycol, and 0.3% gelatin plus 2% sucrose, or other surfactants known to one skilled in the art can be used.

To co-precipitate particles of the compounds in the desired sizes, the precipitating liquid and the solution are combined under controlled conditions of temperature, ratio of

infusion rate to stirring rate, and the proportion of non-solvent to solvent in the dispersed solution.

Preferably, the solution being infused with precipitating liquid is agitated. This can be accomplished by stirring, shaking, by the infusion itself and by other techniques known to those skilled in the art. This effect can also be achieved by combining a stream of precipitating liquid with a stream of the solution.

The co-precipitation of the compounds occurs exothermically, heating the solution and the resulting suspension. The temperature of the solution and resulting suspension is controlled to achieve the particle size of precipitate that is desired. Higher solution temperatures during precipitation produce larger particles, and lower solution temperatures during precipitation produce smaller particles. Since many compounds are less soluble at lower temperatures, it is generally preferred to conduct the infusion of precipitating liquid at a low temperature in order to maximize yield. The lower limit of the temperature at which co-precipitation can be conducted is, of course dependent upon the freezing point of the solvent, precipitating liquid, as well as economic concerns.

Also, faster infusion rates at constant stirring rate of organic solution produce smaller

particles, and slower infusion rates produce larger particles.

FIGS. 6-8 show the effects on particle size of varying parameters during precipitation of IDE from a DMSO solution diluted with 1 part solution to 2 parts ethanol using an aqueous solution of 5% polyvinylpyrrolidone at different infusion rates and temperatures.

FIG. 6 shows that as the volume and stirring rate of the organic compound iodipamide ethyl ester and dimethyl sulfoxide/ethanol solution are increased, the infusion rate of aqueous surfactant solution must be increased proportionally as defined by: infusion rate (ml/min.) = 23 + 0.14 [volume (liters) X stir rate (r.p.m.)] to produce particles of 1 micron diameter at 4°C.

FIG. 7 shows that at a constant ratio of infusion rate to [stir rate X volume], increased precipitation temperature produces larger particles.

FIG. 8 plots 3 points for rate of infusion of the precipitating liquid into the organic solution to approximate the curve by which larger particles are formed from slower injection rates, showing that at a constant ratio of temperature to [stir rate X volume], particle side is inversely related to the rate of infusion of the precipitating liquid.

When FIGS. 6-8 are considered together, they show clearly that higher temperatures and slower mixing rates produce larger particles, and lower temperatures and faster mixing rates produce smaller particles. Another parameter than can be varied to affect particle size is the amount of dilution of the solution before co-precipitation occurs.

When the co-precipitation is complete, extra surfactant solution can be added to further stabilize the suspended particles against agglomeration. The extra solution can be added at a rapid rate, since essentially all the compounds are now co-precipitated in uniformly sized particles. The precipitated particles are promptly separated from the solvent to prevent redissolving and reprecipitation of particles at undesirable sizes. Centrifuging is a preferred way to perform the separation. Other methods, including membrane filtration, reverse osmosis, and others known to persons skilled in the art may also be used to remove undesired substances.

Promptly after separating the particles, the particles are washed or rinsed or titrated with a solution in which one of the two compounds initially dissolved is soluble to remove solvent and excess surfactant and to remove or extract such

solubilized compound from the precipitated particles and dried as discussed hereinabove.

The dried porous particles prepared according to the method outlined above may be resuspended in an appropriate suspension vehicle which may be aqueous or non-aqueous solution, as the situation requires. For example, where the porous particles formed comprise a pharmaceutical compound for parenteral administration, the porous particles are ultimately resuspended in an aqueous solution, such as a sterile saline solution. In so doing, however, gas bubbles, such as air bubbles, will be entrapped in the crevices or pores of the particles. In other instances, the particles may be suspended in a carrying agent such as an ointment, gel, or the like. Preferably, the compound has the same range of solubility in the suspension vehicle as in the precipitating liquid.

It should be understood to those versed in this art that the methods and ultrasmall, non-aggregated solid particles disclosed in U.S. Patents, No. 4,826,689 and No. 4,997,454, provide teachings upon which the instant invention has improved, and therefore are incorporated herein by reference in their entireties.

Examples of ultrasmall, substantially non-aggregated porous particles of the present

invention will now be further illustrated with reference to the following examples.

Example I

Ultrasmall, non-aggregated porous iodipamide ethyl ester (IDE) particles having a substantially uniform mean diameter of about 0.5 microns were prepared as recited hereunder and suspended in bovine plasma-distilled water (1:1) solution and placed in a small plastic pipette.

A pulse echo technique was used to determine relative backscatter. A wide band 10 MHz center frequency, Panametrics transducer (1.3 cm diameter 5 focus), driven by a JSR Pulser, was used to obtain rf scan lines. For in vitro measurements, the mean backscatter (root mean square, RMS) was computed for eight uncorrelated scan lines, each corresponding to about 4 mm in length.

In vitro analysis of the bubble/IDE particle suspension reveals that backscatter increases monotonically with concentration, as shown in FIG. 9.

In vitro analysis of the bubble/IDE particle suspension and a standard solid IDE particle suspension after mixing with bovine plasma, reveals a much higher backscatter from the bubble/IDE particles than from the solid IDE particles, as shown in FIG. 3. Moreover, these data demonstrate

that the high echogenicity of the bubble/IDE particles is sustained for hours after mixing with bovine-plasma.

To prepare the bubble/IDE suspension, about 250 mg of solid iodipamide ethyl ester (IDE) and 500 mg of iodipamic acid (IDA) were added to a 50 ml beaker with 1" x 5/16" teflon coated stir bar. Approximately 5 mls of dimethylsulfoxide (DMSO) were added and stirred for about 10-15 min. until dissolved. To the solution, approximately 6.25 mls of absolute ethanol were added. The beaker was then cooled in an acetone/dry ice bath. While cooling, the mixture was stirred quickly, but without splashing. Approximately 4.5 mls of water at about pH 5 in about 0.5 ml increments were slowly injected into the mixture while maintaining the temperature thereof at about 0°C. Approximately 8 mls of water at about pH 5 was then infused into the mixture at a rate of about 5 mls/min. starting at about 0°C. Thus, a total of 12.5 mls of water was infused into the mixture. A Harvard Infusion pump and a 60 cc plastic syringe and 19 gauge infusion set (butterfly) were used to infuse the water. Co-precipitation occurred after about 6.5 mls of water were infused into the mixture and at a temperature of about 1°C. Following co-precipitation, the suspension was stabilized by adding about 150 mls of

1% polyvinylpyrrolidone (PVP) at about pH 7.4 and kept at a temperature of about 10°C. The suspension was then poured into a 250 ml centrifuge bottle (approximately 31.25 mls) and centrifuged at approximately 2500 rpm for about 30 min. The supernate was discarded. The precipitate in the centrifuge bottle was then repeatedly washed with approximately 31.25 mls of about 0.1% PVP at about pH 11 until the wash reached a pH of about 10 to extract the IDA from the IDE particles to generate the ultrasmall, non-aggregated, porous, non-crystalline IDE particles. The particles where then treated with human serum albumin according to Example III and then lyophyllized according to the procedure set forth in Example IV.

Example II

New Zealand white rabbits (Hazelton Laboratories) weighing 2-4 Kg were anesthetized, and injected intravenously at a rate of about 1 ml/min. with a 8-10 ml (depending on rabbit weight) suspension of iodipamide ethyl ester (IDE) porous particles having a substantially uniform mean diameter of about 0.5 microns. The final concentration of the bubble/IDE particle suspension was approximately 100 mg/ml. The injected dose of the bubble/IDE particle suspension was approximately 250 mg IDE/Kg

rabbit body weight. The IDE particles contained air bubbles trapped within their crevices or pores.

The rabbits were scanned periodically. B-scan images of rabbit liver with and without the bubble/IDE particle suspension are shown in FIGS. 4 and 5. These images were obtained from a 5.0 MHz Acuson Scanner with all settings held constant over a 120 min. examination.

Liver echogenicity following intravenous administration of the bubble/IDE particle suspension is markedly enhanced as compared with no contrast agent, as evidenced by FIGS. 4 and 5. Results in these rabbits demonstrate that the stabilized/ echogenic gas can be delivered to the liver, raising the backscatter well above the levels obtained with no contrast agent or with standard solid IDE particles. Improving liver lesion detection by enhanced ultrasound may now be possible with the bubble/IDE particles.

To prepare the bubble/IDE particle suspension, about 5000 mg of solid iodipamide ethyl ester (IDE) and about 10,000 mg of iodipamic acid (IDA) were added to a 800 ml beaker with 2" X 5/16" teflon coated stir bar. Approximately 100 mls of dimethyl-sulfoxide (DMSO) were added and stirred for about 10-15 min. until dissolved. To the solution, approximately 125 mls of absolute ethanol were

added. The beaker was then cooled in an acetone/dry ice bath. While cooling, the mixture was stirred quickly, but without splashing. Approximately 90 mls of water at about pH 5 at about 10 ml increments were slowly injected into the mixture while maintaining the temperature thereof at about 0°C. Approximately 160 mls of water at about pH 5 was then infused into the mixture at a rate of 100 ml/min. starting at about 0°C. Thus, a total of 250 mls of water was infused into the mixture. A Harvard Infusion pump and a 60 cc plastic syringe and 19 gauge infusion set (butterfly) were used to infuse the water. Co-precipitation occurred after about 125 mls of water were infused into the mixture and at a temperature of about 2°C. Following co-precipitation, the suspension was stabilized by adding 150 mls of about 1% polyvinylpyrrolidone (PVP) at about pH 7.4 and kept at a temperature at about 10°C. The suspension was then poured into five 250 ml centrifuge bottles (approximately 125 ml per bottle) and centrifuged at approximately 2500 rpm for about 30 min. The supernate was discharged. The precipitates remaining in the five centrifuge bottles were then repeatedly washed with approximately 125 mls of about 0.1% PVP at about pH 11 until the wash reached a pH of about 10 to extract the IDA from the particles and to form the

ultrasmall, non-aggregated, porous, non-crystalline IDE particles. The particles were then treated with human serum albumin according to Example III and then lyophyllized according to the procedure set forth in Example IV.

Example III

The IDE particles of Examples I and II were coated with human serum albumin to improve the bio-compatibility of such particles.

To coat the IDE particles, an appropriate amount of the IDE suspension (e.g. 250 mg or 5 g) is pipetted into a 50 ml centrifuge tube. The tube is centrifuged at 2500 rpm for 30 min. and the supernatent discarded. To 5 g or 250 mg of the IDE porous particles, add 100 mls or 5 mls, respectively, of about 25% human serum albumin (HSA) and mix thoroughly with vortex mixer. A preferred HSA/IDE ratio in accordance with the instant invention is about 2:1 to about 5:1. Let the HSA mixture stand at room temperature for about two hours. After standing, mix thoroughly with a vortex mixer approximately every 30 min., and then store at 40°C overnight. Centrifuge the stored mixture at about 3000 rpm for 120 min. Discard supernate. Add by pipette, approximately 2.0 mls for 250 mg of IDE

or 15 mls for 5 g of IDE of about 0.1% PVP at about pH 7.4 and mix thoroughly with vortex mixture.

Example IV

To lyophilize the IDE particles of Examples I or II, place centrifuge tubes in dry ice/acetone bath to freeze suspension as quickly as possible. To facilitate a thin frozen layer, the suspension may be rolled onto the lower sides of the centrifuge tube.

Once frozen, place frozen tubes in lyophilizer as quickly as possible and begin lyophilization. Typical lyophilizer settings include: freeze-dryer temperature equal about -55°C; shelf temperature is equal to about -40°C; and vacuum is at about 10-50 micrometer mercury. The frozen suspension should be lyophilized until the moisture remaining is about 2% or less. When removing from the lyophilizer, immediately cap the centrifuge tubes and store in a refrigerator at about 5°C until needed.

Alternatively, to lyophilize the IDE particles of Examples I or II, a compound such as mannitol, or other suitable agent known to one skilled in the art to facilitate lyophilization cake formation, should be added to the suspending vehicle. Up to about 4 ml of suspension then is

added to about 10 ml lyophilization vials (or equivalent ratio for larger volumes) and flash freeze the vial contents. The vials then are placed in a lyophilizer for vacuum drying. A profile that works includes a condenser of about −55°C and a vacuum of about 10-50 micrometers of mercury. The shelf temperature is adjusted to about −40°C for about 48 hours then increased to about −15°C for about 24 hours followed by about 8 hours at about 20°C. Other profiles could be acceptable as long as the moisture remaining is about 2% or less. The vials should be sealed, removed from the lyophilizer and stored at about 5°C until needed.

<u>Example V</u>

To prepare the 1:6 bubbicles (porous particles) suspension (Example 16, Table I), about 10 grams of iodipamide ethyl ester (IDE) and about 60 grams of iodipamic acid (IDA) were added to a two liter beaker. Approximately two hundred milliliters of dimethylsulfoxide (DMSO) were added to the beaker and stirred for approximately one hour until all of the solids dissolved. About two hundred and fifty milliliters of ethanol was then added to the solution and the solution was mixed. The beaker containing the solution was then placed in a dry ice/acetone bath and stirred quickly while it cooled

to about 10°C. Two hundred and twenty milliliters of water (about pH 5) was then infused at a rate of about 150 ml/min by means of a Harvard Infusion pump, about 60 cc plastic syringe, and 19 gauge butterfly set. The water (about pH 5) was infused in about twenty milliliter increments into the stirring beaker, allowing the temperature to return to about 10°C after each increment. The solution in the beaker was then cooled to about 8°C and about 60 milliliters of water (about pH 5) was infused at a rate of about 100 ml/min, so that the precipitation would occur at a total infused volume of about 250 ml and a temperature of about 9°C. An additional 220 ml of water (about pH 5) was then infused at a rate of about 150 ml/min into the beaker. The co-precipitated bubbicles (porous particles) were then stabilized by the addition of about 300 ml of 1% PVP (about pH 7.4).

The material was then poured into six 250 ml centrifuge bottles (about 208 ml per bottle) and centrifuged at about 2500 rpm for about 45 minutes. The supernatant was discarded and each bottle was resuspended in about 125 ml of about 0.1% PVP (about pH 11). The bottles were then centrifuged (about 2500 rpm for about 30 minutes) twice more and resuspended in both about 125 ml of about 0.1% PVP (about pH 11) per bottle and finally about 50 ml of

about 0.1% PVP (about pH 11) per bottle. About one hundred and twenty five milliliters of about 0.1 N sodium hydroxide was then added to each bottle. The bottles were then washed eleven times by centrifuging at about 2500 rpm for about 30 minutes and resuspending with about 0.1% PVP (about pH 7.4). Once all of the material was washed, the material was again centrifuged and resuspended in about 0.1% PVP, about 10% mannitol (about pH 7.4), diluted with the mannitol solution to about 50 mg/ml, and lyophilized in doses of 4 ml per 10 ml vial as described in Example IV.

Table I hereinafter illustrates the production of ultrasmall porous IDE particles in accordance with Examples I and V. More particularly, Examples 6-10 in Table I generally follow the procedure outlined in Example I, whereas Examples 11-14 in Table I also follow Example I procedures generally, but illustrate a variation in the IDE/IDA ratio. Example 15, as compared to Example 13, in Table I shows how the diameter of the porous particles can be increased by increasing temperature. Examples 15 and 16 demonstrate how porous particles production can be scaled up while maintaining constant particle diameter.

TABLE I

Example No.	6	7	8	9	10	11	12	13	14	15	16
IDE (grams)	0.5	1.0	2.0	0.5	0.75	1.0	1.0	1.0	1.0	1	10
IDA (grams)	1.0	2.0	4.0	1.0	1.5	1.0	4.0	6.0	8.0	6	60
DMSO (ml)	10	20	40	5	5	20	20	20	20	20	200
ETOH (ml)	12.5	25	50	6.25	6.25	25	25	25	25	25	250
Precipitating Solution (ml)	25	50	100	12.5	12.5	50	50	50	50	50	500
Infusion Rate (ml/min)	10	20	40	5	5	100	100	20	100	20	150
Temperature (°C)	1	0	1.5	23	24.5	6	1	-10	5	11.5	8.5
Bubbicle Size (nm)	584	572	619	1240	1620	489	459	571	513	1055	1019

Exs. 6-8 Demonstrate scaleup of the 1:2 porous particles preparation at the 0.5 micron diameter size.

Exs. 6,9,10 Demonstrate how particle size is increased by increasing the concentration and temperature of precipitation.

Exs. 11-14 Demonstrate varying IDE/IDA ratios while maintaining 0.5 micron diameter.

Exs. 15-16 Demonstrate scaleup of the 1:6 porous particles preparation at the 1 micron diameter size.

The present invention may, of course, be carried out in other specific ways than those herein set forth without departing from the spirit and essential characteristics of the invention. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive and all changes coming within the mean and equivalency range of the appended claims are intended to be embraced therein.

Having described our invention, we claim:

(1) Ultrasmall, substantially non-aggregated, non-crystalline porous particles having substantial uniformity in size selected from a particle diameter range of up to about 10 microns, said particles having the ability to trap gas bubbles within its porous matrix when suspended in a liquid and to enhance medical images of blood vessels and soft tissue when introduced into the blood stream of an animal.

(2) Ultrasmall porous particles according to claim 1 wherein the mean particle diameter is selected from the range of from about 0.01 microns to about 5.0 microns.

(3) Ultrasmall porous particles according to claim 1 wherein the mean particle diameter is selected from the range of from about 0.1 microns to about 2.0 microns.

(4) Ultrasmall porous particles according to claim 1, said particles being porous iodipamide ethyl ester particles.

(5) Ultrasmall porous particles according to claim 1, said particles being suspended in a liquid and having gas bubbles entrapped within their porous matrices.

(6) Ultrasmall porous particles according to claim 1, said particles having a coating on their surfaces.

(7) Ultrasmall, substantially non-aggregated, non-crystalline porous particles having substantial uniformity in size selected from a particle diameter range of up to about 10 microns, said particles being produced by the process comprising:

(a) preparing a solution which includes first and second solid compounds in a suitable solvent for the two compounds wherein;

(b) infusing a precipitating liquid into the solution at a temperature between about -50°C and about 100°C at an infusion rate of from about 0.01 ml/min. to about 3000 ml/min. per 50 ml unit volume of solution, the two solid compounds having essentially little solubility in the precipitating liquid and the solvent being miscible in the precipitating liquid, so as to produce a suspension of precipitated amorphous, non-crystalline solid compounds in the form of substantially non-aggregated particles of a uniform size selected from a particle diameter range of up to about 10 microns, the particle size being directly related to the solution temperature during precipitation and inversely related to the infusion rate; and

(c) washing-the particles in a suitable washing liquid, the first compound having essentially little solubility in the washing liquid and the second compound being soluble in said washing liquid, so that the second compound is extracted from the first compound to produce the ultrasmall, substantially non-aggregated, non-crystalline porous particles.

(8) Ultrasmall porous particles according to claim 7, said process further including the step of:

separating the co-precipitated particles from the solvent before said washing or during said washing.

(9) Ultrasmall porous particles according to claim 7, wherein additional precipitating liquid is added to the suspension before the particles are washed.

(10) Ultrasmall porous particles according to claim 8, wherein the particles are separated by centrifugation, membrane filtration, or reverse osmosis.

(11) Ultrasmall porous particles according to claim 7, wherein the precipitating liquid is a surfactant solution.

(12) Ultrasmall porous particles according to claim 7, wherein a solution is prepared such that the concentration of the two solid compounds are near their solubility limits in the solvent.

(13) Ultrasmall porous particles according to claim 7, wherein the first solid compound is iodipamide ethyl ester and the second solid compound is iodipamic acid, the solvent is DMSO, the precipitating liquid is water at a pH of about 5.0, and the washing liquid is an aqueous solution of polyvinylpyrrolidone at a pH of about 11.

(14) Ultrasmall porous particles according to claim 7, wherein the mean particle diameter is selected from the range of from about 0.01 microns to about 5.0 microns.

(15) Ultrasmall porous particles according to claim 7, wherein the mean particle diameter is selected from the range of from about 0.1 microns to about 2.0 microns.

(16) Ultrasmall porous particles according to claim 7, wherein the particle size distribution has a maximum relative standard deviation of about 40%.

(17) Ultrasmall porous particles according to claim 7 wherein the weight ratio of the second compound soluble in the washing fluid to the first compound insoluble in the washing fluid is in the range of from about 2:1 to about 10:1.

(18) Ultrasmall porous particles according to claim 7, said process including the further step of drying said ultrasmall porous particles.

(19) Ultrasmall porous particles according to claim 7, said ultrasmall porous particles being suspended in a liquid and having gas or a medically useful compound entrapped within their porous matrices.

(20) Ultrasmall porous particles according to claim 7, said process including the further step of coating said ultrasmall porous particles.

(21) A sterile injectible fluid composition of matter in unit dosage form and adapted for injection into the blood stream of an animal for providing gas bubble echogenicity for blood vessels and soft tissue ultrasound imaging, said composition comprising a suspension of said ultrasmall porous particles as recited in claim 1 suspended in a carrier liquid which is non-toxic and physiologically acceptable and in which the ultrasmall porous particles are at least temporarily stable.

(22) A composition of matter according to claim 21 wherein the ultrasmall porous particles are iodipamide ethyl ester particles.

(23) A composition of matter according to claim 22 wherein the iodipamide ethyl ester particles are substantially uniform in size having a mean particle diameter size of about 0.5 microns.

(24) A composition of matter according to claim 21 wherein said ultrasmall porous particles are coated.

(25) A method for enhancing ultrasound imaging in the blood vessels and soft tissue of an animal, said method comprising

introducing the composition of matter as recited in claim 21 into an animal in an effective amount; and

scanning the animal with an ultrasound scanner to generate an ultrasound image.

(26) A method of forming ultrasmall, substantially non-aggregated, non-crystalline porous particles having substantial uniformity in size selected from a particle diameter range of up to about 10 microns, said method comprising:

(a) preparing a solution which includes first and second solid compounds in a suitable solvent for the two compounds;

(b) infusing a precipitating liquid into the solution at a temperature between about -50°C and about 100°C at an infusion rate of from about 0.01 ml/min. to about 3000 ml/min. per 50 ml unit volume of solution, the two solid compounds have an essentially little solubility in the precipitating liquid and the solvent being miscible in the precipitating liquid, so as to produce a suspension of precipitated amorphous, non-crystalline solid compounds in the form of substantially non-aggregated particles of a uniform size selected from a particle diameter range of up to about 10 microns, the particle size being directly related to the solution temperature during precipitation and inversely related to the infusion rate; and

(c) washing the particles in a suitable washing liquid, the first compound having essentially little solubility in the washing liquid and the second compound having greater solubility in said washing liquid, so that the second compound is extracted from the first compound to produce the ultrasmall, substantially non-aggregated, non-crystalline porous particles.

(27) A method according to claim 26 including the further step of separating the co-precipitated particles from the solvent before said washing.

(28) A method according to claim 26 including the further step of drying said ultrasmall porous particles.

(29) A method according to claim 26 including the further step of coating said ultrasmall porous particles.

(30) A method according to claim 26 including the further step of suspending the ultrasmall porous particles in a liquid.

(31) A method according to claim 26 including the further step of introducing a gas or a medically useful compound into said ultrasmall porous particles.

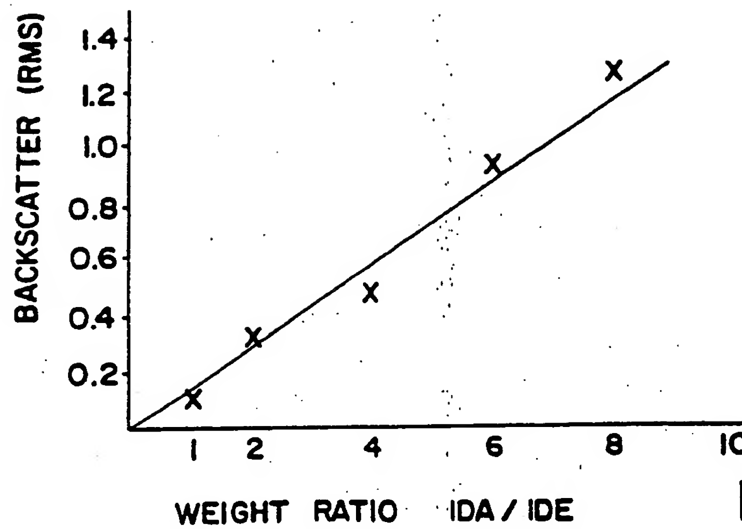

FIG. 1

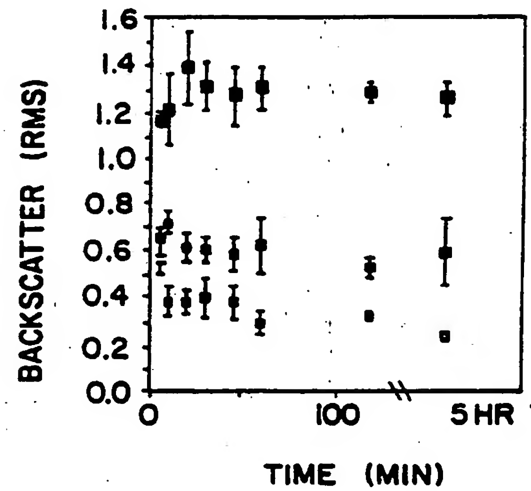

FIG. 2

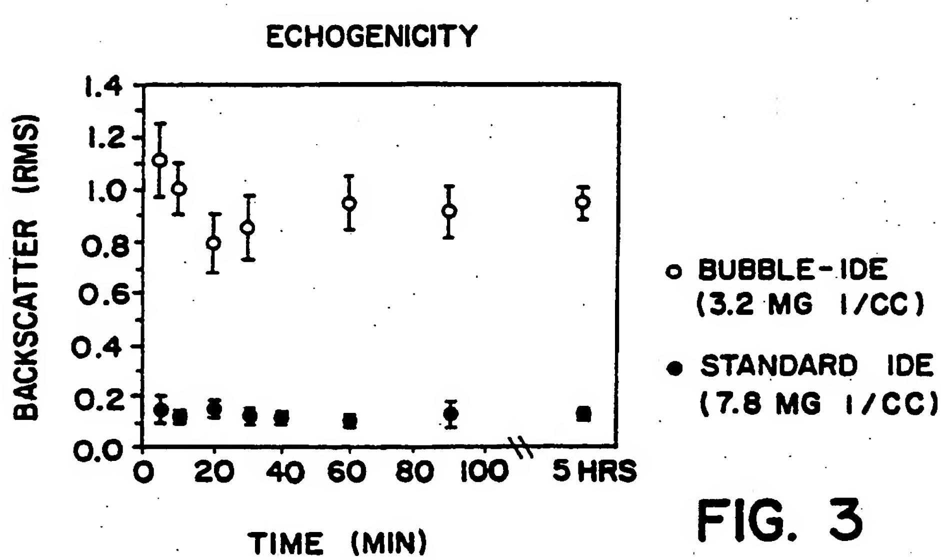

FIG. 3

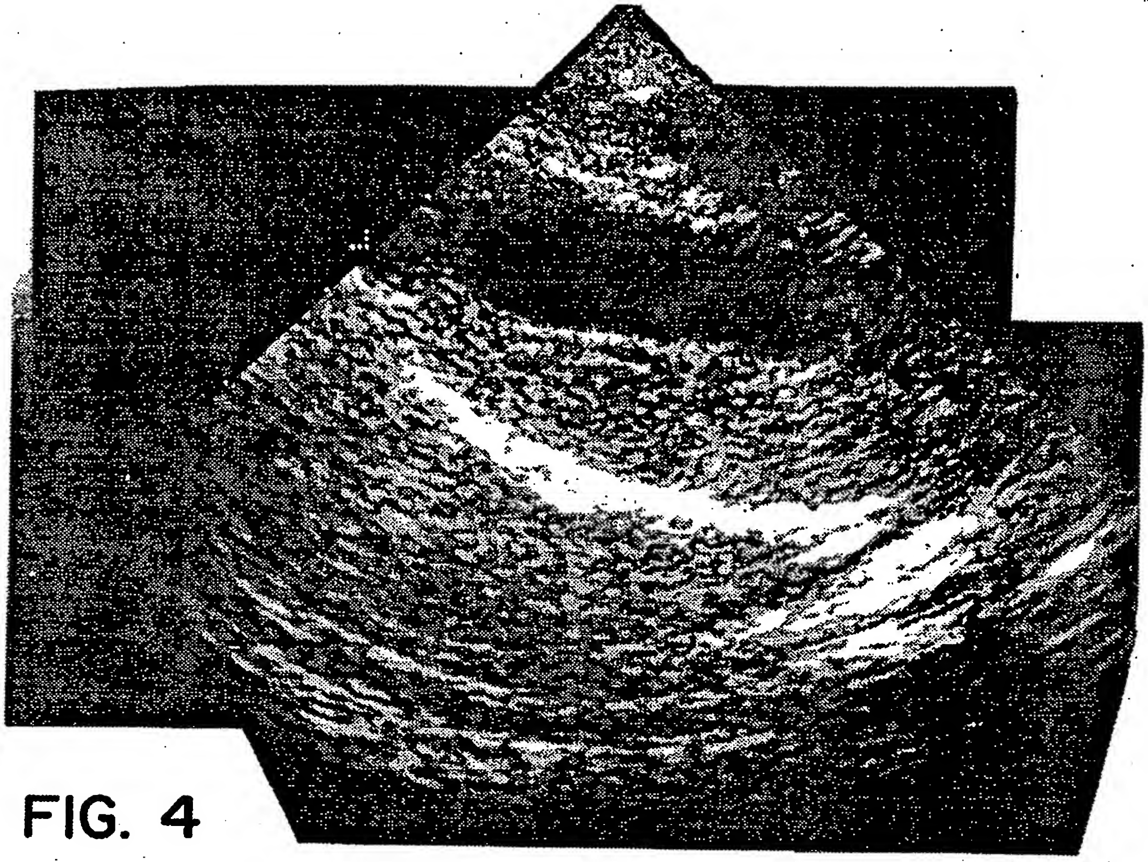

FIG. 4

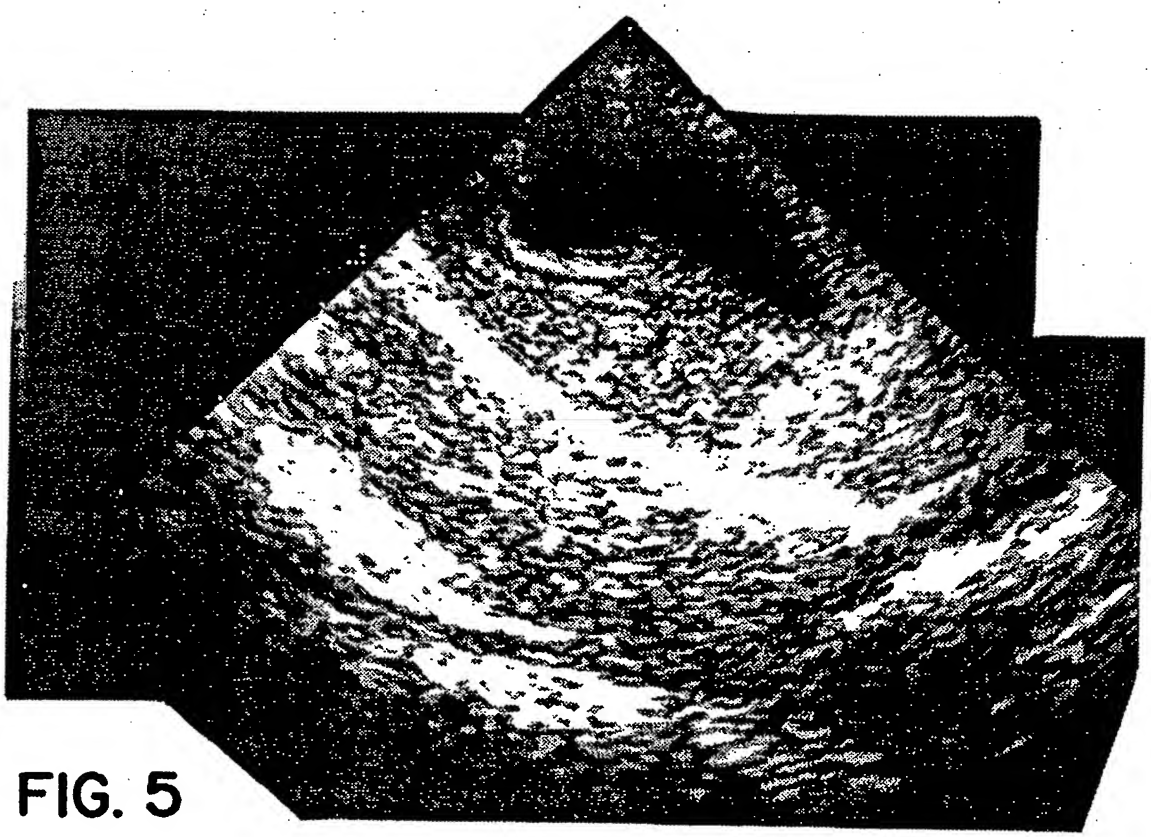

FIG. 5

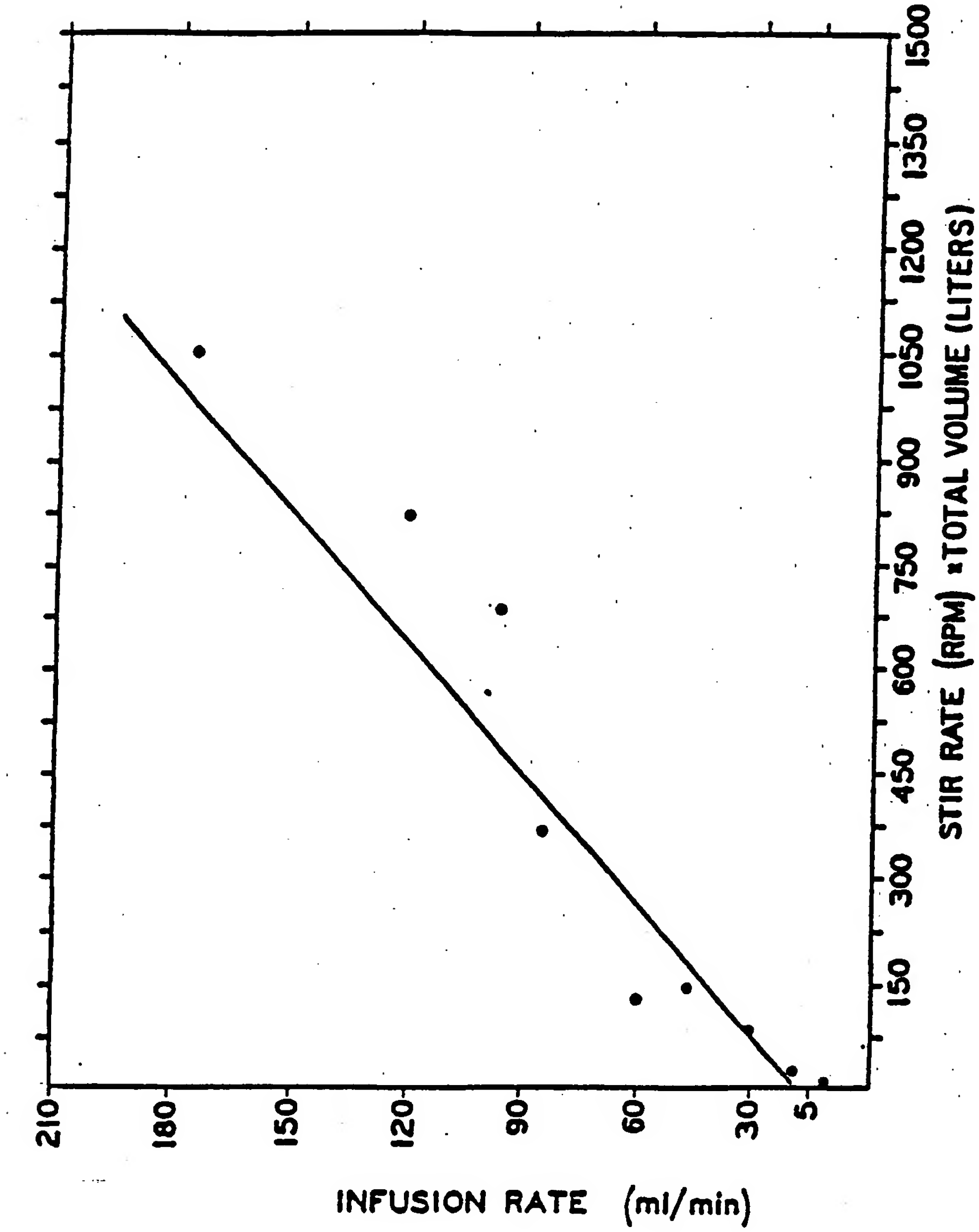
STIR RATE (RPM) x TOTAL VOLUME (LITERS)
150
300
450
600
750
900
1050
1200
1350
1500
INFUSION RATE (ml/min)
210
180
150
120
90
60
30
5

FIG. 6

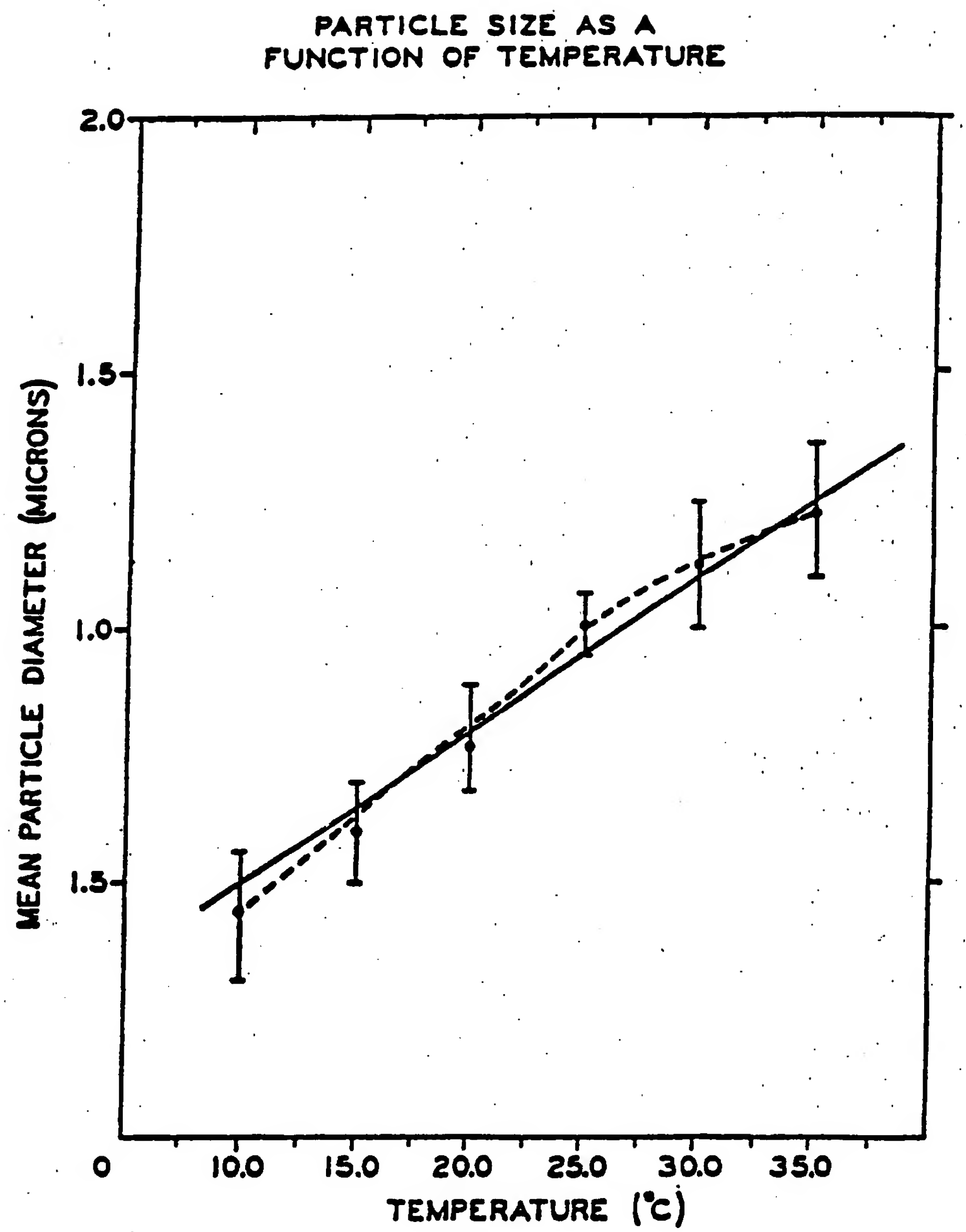
PARTICLE SIZE AS A
FUNCTION OF TEMPERATURE
2.0
1.5
1.0
1.5
MEAN PARTICLE DIAMETER (MICRONS)
0
10.0
15.0
20.0
25.0
30.0
35.0
TEMPERATURE (°C)

FIG. 7

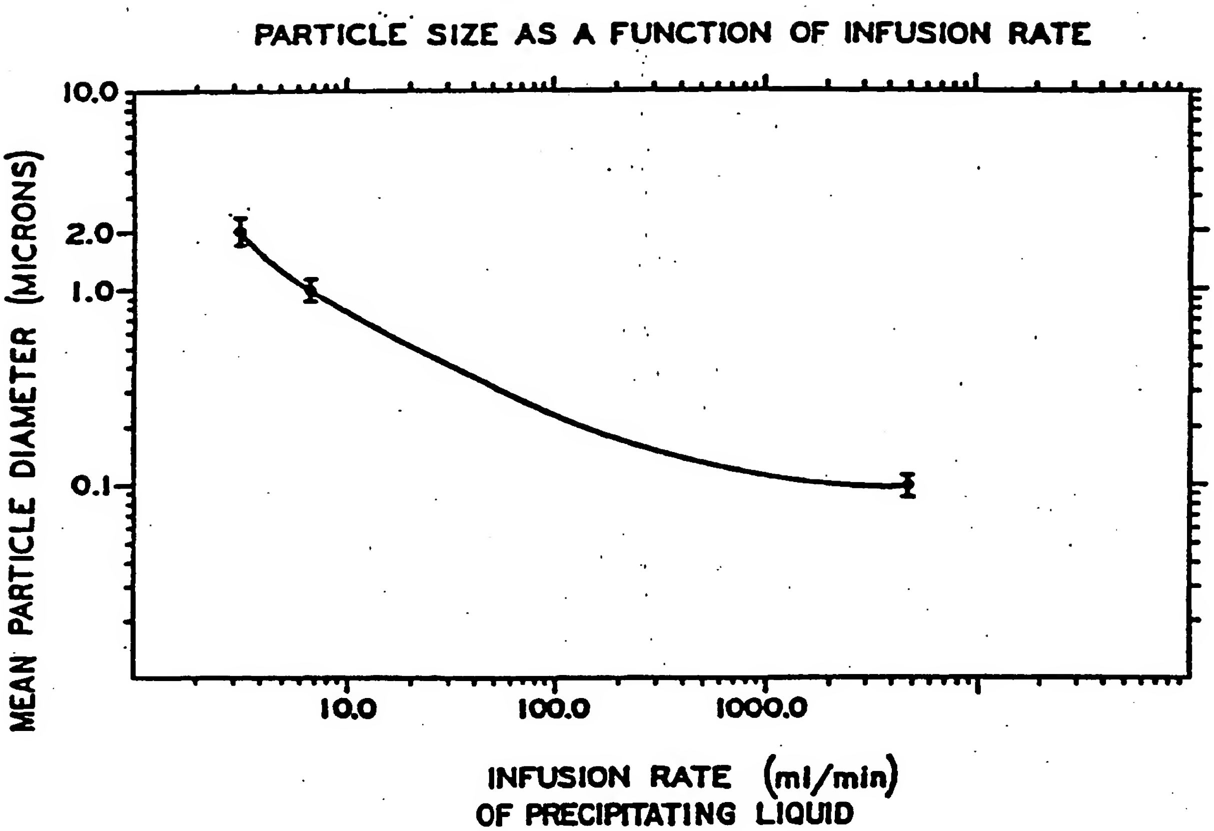
PARTICLE SIZE AS A FUNCTION OF INFUSION RATE
MEAN PARTICLE DIAMETER (MICRONS)
10.0
2.0
1.0
0.1
10.0
100.0
1000.0
INFUSION RATE (ml/min)
OF PRECIPITATING LIQUID

FIG. 8

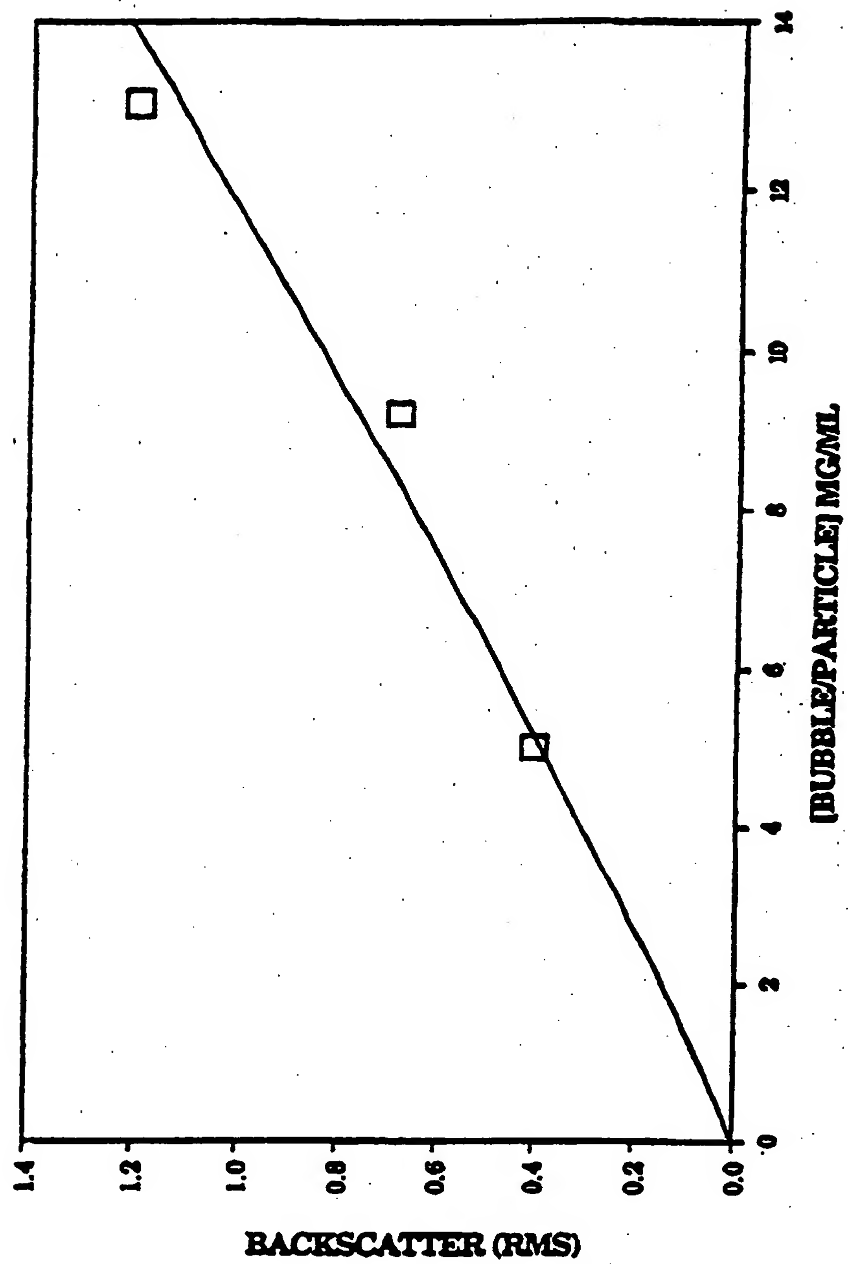
BACKSCATTER (RMS)
[BUBBLE/PARTICLE] MG/ML
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
2
4
6
8
10
12
14

FIG. 9

International Application No PCT/US 92/05592

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 49/00 A 61 K 49/04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	EP,A,0169618 (STERILIZATION TECHNICAL SERVICES) 29 January 1986, see page 4, line 23 - page 5, line 19; page 6, lines 1-8; table If; page 29, example 29 ---	1-31
Y	EP,A,0052575 (ULTRA MED. INC.) 26 May 1982, see page 10, lines 11-28; page 41, line 9 (cited in the application) ---	1-31
Y	EP,A,0414287 (NYCOMED S.A.) 27 February 1991, see page 3, line 28 - page 4, line 10; page 4, lines 34-39,51-56 --- -/-	1-31

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
23-09-1992	02. 11. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Dagmar Frank

Mme Dagmar FRANK

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	STN File Server, File Medline, AN=92225855, M.R. VIOLANTE et al.: "Particle-stabilized "bubbles" for enhanced organ ultrasound imaging", & INVEST. RADIOL., (1991 NOV) 26 SUPPL. 1 S194-7, DISCUSSION S198-200, see whole abstract ---	1-31
A	DE,A,3246386 (M.W. HELZEL) 20 June 1984, see claims -----	1-31

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9205592
SA 62092

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 20/10/92
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0169618	29-01-86	AU-B- 579166	17-11-88
		AU-A- 4501285	22-01-87
		CA-A- 1282405	02-04-91
		JP-A- 62027032	05-02-87
		PT-A- 80494	22-11-85
		WO-A- 9203380	05-03-92
		US-A- 4997454	05-03-91
		US-A- 4826689	02-05-89
EP-A- 0052575	26-05-82	AU-B- 565842	01-10-87
		AU-A- 4127485	19-09-85
		AU-B- 545866	01-08-85
		AU-A- 7931382	07-06-82
		CA-A- 1170569	10-07-84
		WO-A- 8201642	27-05-82
		US-A- 4442843	17-04-84
		US-A- 4657756	14-04-87
		US-A- 4681119	21-07-87
EP-A- 0414287	27-02-91	AU-A- 6163390	22-02-91
		WO-A- 9101147	07-02-91
		EP-A- 0483278	06-05-92
DE-A- 3246386	20-06-84	None	

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82